Amendment to the Specification

Please amend the paragraph beginning at page 9, line 9, as follows:

Because normal mature B cells also express CD20, normal B cells are depleted during CD20 antibody therapy (Reff, M. E. et al, Blood 83: 435-445, 1994). However, after treatment is completed, normal B cells are regenerated from CD20 negative B cell precursors; therefore, patients treated with anti-CD20 therapy do not experience significant immunosuppression. Depletion of normal B cells may be beneficial in diseases that involve inappropriate production of autoantibodies or other diseases where B cells may play a role. A chimeric mAb specific for CD20, consisting of heavy and light chain variable regions of mouse origin fused to human IgG1 heavy chain and human kappa light chain constant regions, retained binding to CD20 and the ability to mediate ADCC and to fix complement (Liu et al., J. Immunol. 139:3521-26 (1987); Robinson et al., U.S. Pat. No. 5,500,362). This work led to development of a chimeric CD20 mAb, RITUXIMAB™ ~~Rituximab™~~, currently approved by the U.S. Food and Drug Administration for approval for therapy of B cell lymphomas. While clinical responses are frequently observed after treatment with RITUXIMAB™ ~~Rituximab™~~, patients often relapse after about 6-12 months.

Please amend the paragraph beginning at page 9, line 23, as follows:

High doses of RITUXIMAB™ ~~Rituximab™~~ are required for intravenous injection because the molecule is large, approximately 150 kDa, and diffusion is limited into the lymphoid tissues where many tumor cells reside. The mechanism of anti-tumor activity of RITUXIMAB™ ~~Rituximab™~~ is thought to be a combination of several activities, including ADCC, fixation of complement, and triggering of signals in malignant B cells that promote apoptosis. The large size of RITUXIMAB™ ~~Rituximab™~~ prevents optimal diffusion of the molecule into lymphoid tissues that contain malignant B cells, thereby limiting these anti-tumor activities. As discussed above, cleavage of CD20 mAbs with proteases into Fab or $F(ab')_2$ fragments makes them smaller and allows better penetration into lymphoid tissues, but the effector functions important for anti-tumor activity are lost. While CD20 mAb fragments may be more effective than intact antibody for delivery of radioisotopes, it would be desirable to construct a CD20 mAb derivative that retains the

effector functions of the Fc portion, but is smaller in size, facilitating better tumor penetration and resulting in a shorter half-life.

Please amend the paragraph beginning at page 51, line 16 as follows:

Typically, the constructs are derived from plasmid vectors. A preferred construct is a modified pNASS vector (Clontech, Palo Alto, CA), which has nucleic acid sequences encoding an ampicillin resistance gene, a polyadenylation signal, and a T7 promoter site. Other suitable mammalian expression vectors are well known (see e.g., Ausubel et al., 1995, Sambrook et al., supra; see also, e.g., catalogues from Invitrogen, San Diego, CA; Novagen, Madison, WI; Pharmacia, Piscataway, NJ; and others). Presently preferred constructs may be prepared that include a dihydrofolate reductase (DHFR) encoding sequence under suitable regulatory control, for promoting enhanced production levels of the binding-domain immunoglobulin fusion protein ~~protei~~, which levels result from gene amplification following application of an appropriate selection agent (e.g., ~~methetrexate~~ methotrexate).

Please amend the paragraph beginning at page 65, line 23, as follows:

The scFv-Ig was assembled by inserting the 2H7 scFv HindIII-BclI fragment into pUC 19 containing the human IgG1 hinge, CH2, and CH3 regions, which was digested with restriction enzymes, HindIII and Bcl1. After ligation, the ligation products were transformed into DHSα bacteria. Positive clones were screened for the properly inserted fragments using the SacI site at the V_L-V_H junction of 2H7 as a diagnostic site. The 2H7seFv-Ig eDNA was subjected to cycle sequencing on a PE 9700 thermocycler using a 25-cycle program by denaturing at 96° C for 10 seconds, annealing at 50° C for 30 seconds, and extending at 72° C for 4 minutes. The sequencing primers were pUC forward and reverse primers and an internal primer that annealed to the CH2 domain human in the IgG constant region portion. Sequencing reactions were performed using the ~~Big Dye~~ BIG DYE™ Terminator Ready Sequencing Mix (PE-Applied Biosystems, Foster City, Calif.) according to the manufacturer's instructions. Samples were subsequently purified using ~~Centrisep~~ CENTRISEP columns (Catalog # CS-901, Princeton Separations, Adelphia, N.J.), the eluates dried in a Savant vacuum dryer, denatured in Template Suppression Reagent (PE-ABI), and analyzed on an ABI 310 Genetic Analyzer (PE-Applied Biosystems). The sequence was

edited, translated, and analyzed using Vector ~~Nti~~ NTI™ version 6.0 (Informax, North Bethesda, Md.). Figure 1 shows the cDNA and predicted amino acid sequence of the 2H7scFv-Ig construct.

Please amend the paragraph beginning at page 68, line 4, as follows:

Supernatants were collected from CHO cells expressing the 2H7scFv-Ig, filtered through 0.2 μm PES express filters (Nalgene, Rochester, N.Y.) and were passed over a Protein A-agarose (IPA 300 crosslinked agarose) column (Repligen, Needham, Mass.). The column was washed with PBS, and then bound protein was eluted using 0.1 M citrate buffer, pH 3.0. Fractions were collected and eluted protein was neutralized using 1M Tris, pH 8.0, prior to dialysis overnight in PBS. Concentration of the purified 2H7scFv-Ig (SEQ ID NO:15) was determined by absorption at 280 nm. An extinction coefficient of 1.77 was determined using the protein analysis tools in the Vector ~~Nti~~ NTI™ Version 6.0 Software package (Informax, North Bethesda, Md.). This program uses the amino acid composition data to calculate extinction coefficients.

Please amend the paragraph at page 73, line 5, as follows:

The 2H7scFv-CD154 construct cDNAs were subjected to cycle sequencing on a PE 9700 thermocycler using a 25-cycle program that included denaturating at 96° C, 10 seconds, annealing at 50° C for 5 seconds, and extension at 60° C, for 4 minutes, The sequencing primers used were pD18 forward (SEQ ID NO: 30: 5'-gtctatataagcagagctctggc-3') and pD18 reverse (SEQ ID NO: 31: 5'-cgaggctgatcagcgagctctagca-3') primers. In addition, an internal primer was used that had homology to the human CD154 sequence (SEQ ID NO: 32: 5'-ccgcaatttgaggattctgatcacc-3'). Sequencing reactions included primers at 3.2 pmol, approximately 200 ng DNA template, and 8 μl sequencing mix. Sequencing reactions were performed using the ~~Big Dye~~ BIG DYE™ Terminator Ready Sequencing Mix (PE-Applied Biosystems, Foster City, Calif.) according to the manufacturer's instructions. Samples were subsequently purified using ~~Centrisep~~ CENTRISEP columns (Princeton Separations, Adelphia, N.J.). The eluates were dried in a Savant speed-vacuum dryer, denatured in 20 .μl template Suppression Reagent (ABI) at 95° C for 2 minutes, and analyzed on an ABI 310 Genetic Analyzer (PE-Applied Biosystems). The sequence was edited, translated, and analyzed using Vector ~~Nti~~ NTI™ version 6.0 (Informax, North Bethesda, Md.). The

2H7scFv-CD154 L2 cDNA sequence and predicted amino acid sequence is presented in Figure 7A, and 2H7scFv-CD154 S4 cDNA sequence and predicted amino acid sequence is presented in Figure 7B.

Please amend the paragraph beginning at page 75, line 11, as follows:

Purified fusion protein derivatives of CytoxB-scFvIg molecules were analyzed by SDS-PAGE according to the methods described in Example 2. Polyacrylamide gels were run under non-reducing and reducing conditions. Two different molecule weight marker sets, BIO RAD ~~BioRad~~ prestained markers, (BioRad, Hercules, Calif.) and Novex ~~Multimark~~ MULTIMARK™ molecular weight markers were loaded onto each gel. The migration patterns of the different constructs and of RITUXIMAB™ ~~Rituximab™~~ are presented in Figure 12.

Please amend the paragraph beginning at page 75, line 17, as follows:

The ability of the different derivatives of CytoxB-scFvIg molecules to mediated ADCC was measured using the Bjab B lymphoma cells as the target and freshly prepared human PBMCs as effector cells, (See Example 2). Effector to target ratios were varied as follows: 70:1, 35:1, and 18:1, with the number of Bjab cells per well remaining constant but the number of PBMCs were varied. Bjab cells were labeled for 2 hours with ^{51}Cr and aliquoted at a cell density of 5×10^4 cells/well to each well of flat-bottom 96 well plates. Purified fusion proteins or RITUXIMAB™ ~~rituximab~~ were added at a concentration of 10 mg/ml to the various dilutions of PBMCs. Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 4 hours, and 100 µl of culture supernatant was harvested to a LUMAPLATE™ ~~Lumaplate~~ (Packard Instruments) and allowed to dry overnight prior to counting cpm released. The results are presented in Figure 13.